indeed fixed by counting subunits in many individual complexes using TIRF microscopy (Ulbrich and Isacoff, 2007, 2008). We expressed GFP-tagged KCNQ1 or KCNE1 in *Xenopus* oocytes at low density and counted bleaching steps in many fluorescent spots corresponding to single channel complexes. First, we confirmed that KCNQ1 forms a tetramer. Next we counted GFP-tagged KCNE1 subunits co-expressed with mCherry-tagged KCNQ1. We observed up to four bleaching steps from GFP-KCNE1 co-localized with mCherry, indicating that up to four KCNE1 subunits can bind to one KCNQ1 tetrameric channel. We find that the number of KCNE1 subunits per complex increases as the expression of KCNE1 is raised relative to that of KCNQ1. Our results suggest that modulation of KCNQ channels may be regulated by the level of expression of KCNE subunits.

708-Pos

Structural Underpinnings for Modulation of the Voltage-Gated Potassium Channel KCNQ1 by the KCNE Family of Proteins

Wade D. Van Horn, Congbao Kang, Carlos G. Vanoye, Richard C. Welch, Alfred L. George, Charles R. Sanders.
Vanderbilt University, Nashville, TN, USA.

The voltage-gated potassium channel KCNQ1 is modulated by KCNE1 to form the I_{Ks} current involved in cardiac repolarization. KCNE1 is the best characterized member of a family of modulatory proteins which impart distinct ion channel physiology. Mutations in KCNQ1 and familial KCNE proteins have been linked to human diseases including congenital deafness and congenital long QT syndrome, which is an inherited predisposition to potentially life-threatening cardiac arrhythmias. The biophysical basis of the KCNE1 modulation of KCNQ1 has been previously characterized in our lab with an interdisciplinary approach utilizing nuclear magnetic resonance (NMR) spectroscopy, electrophysiology, biochemistry, and computational biology. In this work we extend the characterization to include two other family members; namely, KCNE3 and KCNE4. KCNQ1 homology models and the KCNE family proteins KCNE1, KCNE3, and KCNE4 are used as the basis of a comparative study to deduce the molecular mechanisms of voltage-gated potassium channel regulation by these accessory subunits. KCNE1 binds to KCNQ1 and causes delayed channel activation and increased conductance, while, KCNE3 promotes rapid and increased conductance in KCNQ1. On the other hand, KCNE4 binding causes a strict inhibition of KCNQ1 conductance. In this work we present data that suggests the structural biological basis for how the homologous KCNE1, KCNE3, and KCNE4 proteins modulate KCNQ1 in such starkly contrasting manners. *This work was supported by NIH grant R01DC007416.*

709-Pos

KCNE4 Juxtamembrane Region Interacts with Calmodulin and is Necessary for KCNQ1 Modulation

Erin J. Ciampa, Carlos G. Vanoye, Alfred L. George Jr.
Vanderbilt University, Nashville, TN, USA.

Voltage-gated potassium (K_V) channels are modulated by the KCNE family of single transmembrane proteins. A membrane-based yeast two-hybrid screen to discover KCNE4 interacting proteins identified calmodulin (CaM) as a candidate. Previous studies demonstrated that CaM binding to KCNQ1 is required for functional expression of KCNQ1-KCNE1 channels *in vitro*, and increasing concentrations of intracellular calcium stimulate KCNQ1-KCNE1 channels in *Xenopus* oocytes in the presence of wild-type CaM but not mutant CaM that cannot bind calcium. We have tested the functional consequences of the interaction between KCNE4 and CaM with the hypothesis that KCNE4 modulation of K_V currents may depend on its interaction with CaM. We validated the biochemical interaction between KCNE4 and CaM using CaM-agarose pull-down, and tested KCNE4 mutants that targeted putative CaM binding sites. Mutation of a juxtamembrane site (L[69-72]A) exhibited near complete disruption of CaM binding, whereas biotinylation studies performed in CHO cells confirmed expression of the mutant protein at the cell surface. The ability of L[69-72]A to modulate KCNQ1 was then studied using whole-cell patch clamp recording to determine if functional consequences accompany the loss of CaM binding. Wild-type KCNE4 completely inhibits potassium current in CHO cells transiently co-transfected with KCNQ1, but cells co-expressing KCNQ1 with L[69-72]A exhibited KCNQ1-like currents. Mean ($\pm$ SEM) current density (measured during step to +60 mV from holding potential of −80 mV) in cells expressing KCNQ1 alone was 37.0 $\pm$ 4.25, not significantly different from cells co-expressing KCNQ1 with L[69-72]A (32.1 $\pm$ 3.3), but significantly different from cells co-expressing KCNQ1 with wild-type KCNE4 (3.3 $\pm$ 0.35). These studies suggest that a juxtamembrane region in KCNE4 is critical for its interaction with CaM and is necessary for modulation of KCNQ1.

710-Pos

Molecular Mechanisms Underlying Membrane Potential-Mediated Regulation of Neuronal K_{2P}2.1 Channels

Yifat Segal-Hayoun, Asi Cohen, **Noam Zilberberg**.
Ben Gurion University, Beer Sheva, Israel.

The activity of background K_{2P} channels adjusts the resting membrane potential to enable plasticity of excitable cells. Here we have studied the regulation of neuronal human K_{2P}2.1 (KCNK2, TREK-1) channel activity by resting membrane potential. When heterologously expressed in *Xenopus laevis* oocytes, K_{2P}2.1 currents gradually increased several fold at hyperpolarizing potentials and declined several fold at depolarizing potentials, with a midpoint potential of −60 mV. As K_{2P} channels are not equipped with an integral voltage sensor, we sought extrinsic cellular components that could convert changes in the membrane electrical field to cellular activity that would indirectly modify K_{2P}2.1 currents. K_{2P}2.1 voltage sensitivity was found not to be mediated by the activity of either voltage activated calcium channels, the *Xenopus* voltage sensitive proton channel (Xl-Hv) or the *Xenopus* voltage sensor-containing phosphatase (Xl-VSP). On the other hand, we report that membrane depolarization activated the Gq protein-coupled receptor pathway, in the apparent absence of ligand, resulting in phosphatidylinositol-4,5-bisphosphate (PIP_2) depletion through the action of phospholipase C. Our results suggest a novel mechanism in which an indirect pathway confers membrane potential regulation onto channels that are not intrinsically voltage-sensitive to enhance regulation of neuronal excitability levels. The ability of these proteins to operate without any external ligand enhances plasticity at the single cell level, independent of higher regulatory pathways at the tissue or even the organism levels.

711-Pos

K^+ Channel Interacting Proteins 2, 3 and 4 are Critical Components of Kv4 Channel Complexes in Cortical Pyramidal Neurons

Aaron J. Norris, Nicholas C. Foeger, Jeanne M. Nebonne.
Washington Univeristy, St. Louis, MO 63110, MO, USA.

The rapidly activating and inactivating voltage-gated K^+ (Kv) current, I_A, is critical for many neuronal functions, including repetitive firing and synaptic integration. Previous studies revealed that in cortical pyramidal neurons the majority of I_A is encoded by Kv4.2 and Kv4.3 α-subunits. Little, however, is known about the functional roles of K^+ Channel Interacting Proteins (KChIP) 1, 2, 3, and 4 in the generation of I_A. Biochemical experiments revealed that KChIPs 2, 3 and 4 (2-4) co-immunoprecipitate with Kv4.2 in samples from mouse cortex suggesting roles for these three KChIPs in the generation of functional Kv4-encoded channels in cortical pyramidal neurons. Electrophysiological experiments conducted on cortical pyramidal neurons from mice ($KChIP3^{-/-}$) harboring a targeted disruption of the KChIP3 locus revealed that I_A densities and properties were similar to wild type neurons. Interestingly, in cortical samples from $KChIP3^{-/-}$ mice the protein levels of KChIP 2 and 4 were increased suggesting functional compensation for the loss of KChIP3. Similarly, in $KChIP2^{-/-}$ cortices KChIP3 and 4 protein levels were increased relative to wild type. Concurrently knocking down the expression of KChIPs 2-4 using RNAi constructs targeting each of the three KChIPs induced a reduction in I_A density consistent with roles for KChIPs 2-4 in the generation of native Kv4-encoded I_A channels. In cortical samples from $Kv4.2^{-/-}$ and $Kv4.3^{-/-}$ mice, the protein expression levels of KChIPs 2-4 were decreased. Additionally, in samples from mice lacking both Kv4.2 and Kv4.3 KChIP2-4 proteins were barely detectable. Taken together these results demonstrate that KChIPs 2-4 associate with Kv4.2 and Kv4.3 in cortical neurons, this association stabilizes KChIP proteins and, in addition, that KChIPs 2-4 are critical components of native Kv4 channels in cortical pyramidal neurons.

712-Pos

HMR 1098 is not an Sur Isotype Specific Inhibitor of Sarcolemmal or Heterologous K_{ATP} Channels

Haixia Zhang, Alejandro Akrouh, Harley T. Kurata, Maria Sara Remedi, Colin G. Nichols.
Washington University in St. Louis, St. Louis, MO, USA.

Murine ventricular and atrial K_{ATP} channels contain different sulfonylurea receptors (ventricular K_{ATP} channels are Kir6.2/SUR2A complexes, while atrial K_{ATP} channels are Kir6.2/SUR1 complex). HMR 1098, the sodium salt of HMR 1883 {1-[[5-[2-(5-chloro-*o*-anisamido)ethyl]-2-methoxyphenyl]sulfonyl]-3-methylthiourea}, has been used as a selective sarcolemmal (i.e. SUR2A-dependent) K_{ATP} channel inhibitor. However, specificity for ventricular versus atrial channels has not been examined. We used whole-cell patch-clamp techniques on mouse ventricular and atrial myocytes as well as $^{86}Rb^+$ efflux assays and excised inside-out patch-clamp techniques on Kir6.2/SUR2A and Kir6.2/SUR1 channels heterologously expressed in COSm6 cells. In mouse ventricular myocytes, pinacidil-activated K_{ATP} currents were inhibited